

CLAIMS

1. Biologically active recombinant DNA-derived factor IX protein having a specific activity defined as Clotting activity Antigenic concentration as determined by ELISA

of at least 90% of that of blood-derived factor IX, and free from contamination by poxvirus proteins.

05 2. Biologically active recombinant DNA-derived factor IX protein having a molecular weight of about 57 kilodaltons and free from contamination by precursor factor IX to an extent of more than 10 weight % and by poxvirus proteins.

10 3. Factor IX protein according to Claim 1 which is human factor IX protein or sufficiently similar thereto to be acceptable for infusion into human patients suffering from factor IX deficiency.

4. Factor IX protein according to Claim 2 which is human factor IX protein or sufficiently similar thereto to be acceptable for infusion into human patients suffering from factor IX deficiency.

15 5. A process of preparing factor IX protein as defined in Claim 1, which comprises preparing a recombinant expression vector, said vector not being of the poxvirus family, by linking a factor IX DNA sequence to a promotor sequence effective to express the DNA in a eukaryotic cell and incorporating these DNA sequences in a
20 vector, and introducing the expression vector, in vitro, into eukaryotic cells having post-translational modifying means effective to modify the biologically inactive product of the expression of the DNA into the biologically active factor IX protein.

25 6. A process of preparing factor IX protein as defined in Claim 2, which comprises preparing a recombinant expression vector, said vector not being of the poxvirus family, by linking a factor IX DNA sequence to a promotor sequence effective to express the DNA in a eukaryotic cell and incorporating these DNA sequences in a
30 vector, and introducing the expression vector, in vitro, into eukaryotic cells having post-translational modifying means effective to modify the biologically inactive product of the expression of the DNA into the biologically active factor IX protein.

7. A process according to Claim 5 wherein the factor IX DNA sequence comprises substantially all the cDNA sequence complementary to at least that part of factor IX mRNA which codes for the primary translation product.
- 05 8. A process according to Claim 6 wherein the factor IX DNA sequence comprises substantially all the cDNA sequence complementary to at least that part of factor IX mRNA which codes for the primary translation product.
9. A process according to Claim 7 wherein the DNA sequence
- 10 further comprises a non-coding sequence to the 5'-end of the coding sequence.
10. A process according to Claim 8 wherein the DNA sequence further comprises a non-coding sequence to the 5'-end of the coding sequence.
- 15 11. A process according to Claim 5, wherein the expression vector contains a gene providing a selectable marker for eukaryotic cells into which the vector has been introduced and the cells are selected with the aid of the marker.
12. A process according to Claim 5 wherein the eukaryotic cells
- 20 into which the expression vector is introduced are mammalian cells.
13. A process according to Claim 12 wherein the mammalian cells are liver or kidney cells.
14. A process according to Claim 5 wherein the vector is
- 25 introduced by transfection.
15. A process according to Claim 5 wherein the factor IX DNA is human factor IX DNA.
16. A process according to Claim 5 which further comprises recovering the biologically active factor IX protein from the
- 30 eukaryotic cells and purifying it by affinity chromatography.

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